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SUBJECT OF INVESTIGATION

PHYSICOCHEMICAL STUDIES
ON
THE MICROSOMAL RIBONUCLEOPROTEIN
PARTICLES

RESPONSIBLE INVESTIGATOR

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THE MICROSOMAL RIBONUCLEOPROTEIN PARTICLES

Quarterly Progress

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CONTENT

I. THE PURPOSE OF THIS INVESTIGATION

It has been revealed hitherto by several workers (6) that it is on the microsomal ribonucleoprotein (RNP) particles that amino acids are polymerized to protein or its precursor. In elucidating the physiological role of these RNP particles in protein biosynthesis, there are two principal lines of research; the biochemical and physico-chemical approach. This investigation is chiefly concerned with the latter investigative line.

From such a point of view, we intended to make following observations:

1. To determine what bonds are respondible for RNA-protein bonding in the RNP particles.

2. Determination of the molecular weight and the physico-chemical properties of RNA moiety.

3. Physico-chemical investigation on the protein moiety of the RNP particles.

II. RESULTS OBTAINED TO DATE

1. Preparation of RNA in Cold Concentrated Saline

It has been already reported that the microsomal RNP particles isolated by the method of Littlefield et al (5) are dissociated to RNA and protein moieties when treated with concentrated saline in the cold (8). As shown in our preliminary report (9), this property can be applied to the preparation of RNA, In fact, we can demonstrate that this preparation procedure of using only concentrated saline is very convenient and simple, but its molecular weight estimated by sedimentation-viscosity method was found somewhat lower (about 30 x 104) than that prepared by conventional aqueous phenol method (1) (Cf. the next section), a fact which suggests that the RNA isolated by this method suffered some degradation during the isolation procedure. Hence the RNA of high molecular weight was extracted by aqueous phenol and precipitated by concentrated saline in the cold.

2. Determination of the Molecular Weight and Some Physico-chemical Properties of RNA

In the RNA prepared by phenol method, none or only a trace of DNA was detected by diphenylamine test, protein of 0.5 % or less was found as a contaminant by Lowry's method (6), its maximum and minimum absorbancy being found at 258 and 230 mμ respectively (A230/A258 ≈ 0.47).

This RNA was electrophoretically homogeneous, its ascending and descending mobilities in phosphate-saline buffer (pH = 7.0, μ = 0.1) at 0°C being -14 and -13 x 10^{-5} $cm^2.V^{-1}.sec^{-1}$ respectively. Ultrafugation ($\times$ 10^5 rpm) showed that this RNA is composed of two components at least, their sedimentation constant ($S_{20,w}$) being 28 s and 16 s respectively. Using Huggins' equation, its intrinsic viscosity [η] in 0.02 M phosphate buffer (pH = 7.0) at 25°C was found to be 0.42. Assuming that both components are homologous with regard to frictional properties, the value of [η] and $S_{20,w}$ was applied to Sheraga-Mandelkern equation (7) and the sedimation-viscosity molecular weight of these two components were estimates as 1.2 x 10^6 and 0.6 x 10^6 respectively.

3. Electron-microscopical Observation of RNA Molecules

The RNA solutions were diluted with 2 % ammonium acetate-carbonate buffer (pH = 7.0) to a final concentration of 0.002 - 0.005 %, sprayed onto the thin Collodion film spread on freshly cleaved mica surface and shadow-cast with a platinum-paladium alloy at shadow-height ratio of 5:1 and then reinforced with a thin layer of SiO_2. (If the preparation was made directly on the mica as described by Hall, it was very difficult to strip off the film from the mica surface and so slight modification was adopted.) A Hitachi HU-10 electron_microscope was used with magnification of 1 - 2 x 10^4. If necessary,

polystyrene latex of 880 Å in average diameter was added to the RNA solution as an internal standard.

In preparations made such a technique, RNA molecules of various shape were usually observed, a fact which is in marked contrast to the DNA molecules. Roughly spea king, however, they could be calssified two groups; somewhat elongated, rod-like molecules (950 Å in length, 50 Å in width and 30 Å in height on the average), and smaller ones of rather various shapes (spherical, oval and rhomboid). A tendency of aggregation of the former at the bofder and that of the latter at the centre of the micro droplets were noticed. The latter might be unfolded and transformed to the former by flow of fluid in the microdroplet when the solvent evaporated. But we could observe in some cases either of both forms alone. Moreover, it seems worthy to note that the elongated molecules branched like an arrowhead can be found in the ratio of one to about ten rod-like molecules, as if they are composed of two strands twisted with each other. It seems not so improbable, therefore, that two sorts of molecules exist in our microsomal RNA. Assuming that the specific volume of RNA be 0.55, the molecular weight of ro d-like molecule is obtained as order of $1 - 1.5 \times 10^6$, which agrees fairly well with that of 28 s component estimated by sedimentation and intrinsic viscosity. Probably the smaller, polymorph molecules would correspond to 16 s component.

III. RESEARCH PLAN AT THE NEXT QUARTER

1. Acomplishment of the Experiments Made in the First Quarter Period

Some observations stated above will be repeated and the results will be submitted to publication.

2. The Observation of Hyperchromicity of RNA due to Raised Temperature

Because of our failure in obtaining financial support for the apparatus of optical rotation measurement, accurate study on the secondary structure of RNA and protein moieties becomes impossible. Therefore, change in the absorbancy at 260 mμ of RNA produced by heating will be made and compared with change in its ultracentrifugal sedimentation patterns.

3. Electron-microscopical Observations of RNP Particles

Employing the method similar to that applyed to RNA molecules, electron optical observation will be made on RNP particles and, if possible, their fine structure will be examined by negative staining method(4).

IV. LIST OF REFERENCES

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